

III.10 Cryopreservation of *Ribes*

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1 Introduction

The genus *Ribes* L., the currants and gooseberries, includes more than 150 described species of shrubs which are native throughout northern Europe, Asia, North America, and in mountainous areas of South America and north-west Africa (Brennan 1996). Only about 10 or 12 of these species comprise the primary gene pool from which domesticated currants and gooseberries were developed. The discussion in this chapter will focus on the background and cryopreservation of these economically important species.

Total world *Ribes* acreage (Table 1) has been stable over the past several decades although the breakup of the former Soviet Union greatly increased fruit availability. Black currants, the major crop, are primarily grown for the juice market. They are also valued for production of jams, jellies, liqueurs, such as creme de cassis, for the conversion of white wines to rosé, and as flavorants and colorants for dairy products. Black currant juice has intense flavor, color, high ascorbic acid, and other antioxidant levels which are now becoming recognized for their nutraceutical properties. Poland, the Russian Federation, the United Kingdom and the Scandinavian countries lead the world production in black currants.

Red currants are valued for the fresh market and for the production of preserves and juice. The main red currant producers are Poland, Germany, Holland, Belgium, France, and Hungary. Gooseberries, which are eaten fresh or processed into pies and jams, are primarily grown in Poland, Germany, and Hungary (Brennan 1996). Several species of currants have ornamental qualities for plant habit, flowering, or fall foliage.

Ribes production is negligible in North America because of white pine blister rust, *Cronartium ribicola* Fisch., a disease introduced from Asia, which kills susceptible five-needled white pines (Hummer 2000). *Ribes* and *Pinus* L. subgenus *Strobus* are alternate hosts for this disease. Since the early 1900s, *Ribes* culture has been restricted in parts of the United States in an attempt to curtail white pine blister rust.

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Table 1. World production (MT) of *Ribes*, currants and gooseberries, 1998

Country	Black Currants	Gooseberries
Australia	665	
Austria	15,646	1,634
Azerbaijan	200	
Belgium-Luxembourg	2,500	
Bulgaria	100	
Czech Republic	21,000	8,532
Denmark	3,550	
Estonia	1,000	
Finland	2,592	
France	10,586	
Germany	121,200	70,000
Hungary	12,000	5,457
Ireland	1,000	
Italy	250	
Moldova, Republic of	1,000	1,500
Netherlands	1,600	
New Zealand	1,800	25
Norway	18,000	3,200
Poland	165,000	36,014
Romania	1,000	
Russian Federation	180,000	32,000
Slovakia	3,604	1,409
Switzerland	327	34
Ukraine	18,000	
United Kingdom	19,400	2,500
World	601,519	162,305

FAO, 1999.

1.1 *Ribes* Distribution and Important Species

1.1.1 Taxonomy

Ribes was originally placed in the Saxifragaceae (Vetenant 1799; Engler and Prantl 1891), but more recent taxonomic treatments classify the genus in the family Grossulariaceae because of wholly inferior ovary, totally syncarpous gynoecium, and fleshy fruit (Lamarck and De Candolle 1805; Cronquist 1981; Sinnott 1985).

Early classifications also recognized two genera, *Ribes* and *Grossularia* (Coville and Britton 1908; Berger 1924; Komarov 1971). Numerous infrageneric classifications are proposed for these two genera. Prevalent monographs recognize a single genus, *Ribes* (de Janczewski 1907; Sinnott 1985). Crossability between gooseberry and currant species supports the concept of a single genus (Keep 1962). De Janczewski (1907) subdivided the genus into six subgenera: *Coreosma*, the black currants; *Ribes* (+ *Ribesia*), the red currants; *Grossularia*, the gooseberries; *Grossularioides*, the spiny currants; *Parilla*, the Andean currants; and *Berisia*, the European alpine currants.

The centers of diversity for *Coreosma*, *Ribes*, and *Berisia* include Northern Europe, Scandinavia and the Russian Federation (Jennings et al. 1987); and for *Grossularia* in the Pacific Northwest of North America (Rehder 1986). In addition, several species of black currants with sessile yellow glands are native to South America.

1.1.2 Cytology and Evolution

The basic chromosome number of *Ribes* is $x + 8$ (Zielinski 1953) and all species and cultivars are diploid. The chromosome complement and karyotype are highly uniform (Sinnott 1985) and the chromosomes are 1.5 to 2.5 μm (Darlington 1929). Mitotic and meiotic processes are also highly uniform (Zielinski 1953).

The principal evolutionary pressure in the genus appears to be geographical adaptation (Sinnott 1985). Messinger et al. (1999) examined subgeneric taxa of *Ribes* for restriction site variation in two cpDNA regions. While several infrageneric lineages were strongly supported, *Grossularioides* spp. were unexpectedly united with those from *Grossularia*. *Coreosma* spp. exhibited high divergence and were not monophyletic in the analysis. Messinger et al. (1999) consider two possible, not mutually exclusive, evolutionary scenarios for *Ribes*: (1) a long period of stasis is interrupted by sudden radiation of species; and (2) gene flow due to hybridization as a force for diversification.

1.1.3 Important Taxa

Ribes is cultivated for edible fruit, ornamental plant habit, and bloom. The main economically important crop groupings include the black currants, red and white currants, gooseberries, hybrid berries, and ornamentals. The important species within each of these crop groupings are discussed below.

1.1.3.1 Black Currants

Coreosma, the subgenus for black-fruited currants, has sessile resinous glands. The species of most economic importance is *R. nigrum* L., which is native through northern Europe and central and northern Asia to the Himalayas and includes subsp. *europaeum*, subsp. *scandinavicum*, and *R. nigrum* var. *sibiricum* Wolf (Brennan 1996).

Ribes nigrum is an unarmed, strongly aromatic shrub, growing as tall as 2m (Rehder 1986). The leaves are lobed, up to 10cm per side, glabrous above, slightly pubescent with numerous sessile, aromatic glands beneath; the racemes droop and have 4 to 10 flowers. The flowers have reddish – or brownish-green – campanulate hypanthia and recurved sepals. The whitish petals are about two-thirds as long as the sepals. The fruits are globose, up to 10mm diameter, and are generally shiny black when ripe, although green- and yellow-fruited forms exist (Liberty Hyde Bailey Hortorium 1976). This species

was domesticated within Northern Europe by 1600 and is described in early herbals (Brennan 1996). Recent breeding efforts have doubled fruit size compared to wild fruits. Breeders cross *R. nigrum* with *R. ussuriense* Jancz., *R. dikuscha* Fisch. and *R. nigrum* var. *sibiricum* for disease resistance; with *R. bracteosum* Dougl. for longer racemes resulting in higher yield (Brennan 1996). *Ribes hudsonianum* Rich., the northern North American black currant, and *R. americanum* Mill., the American black currant, have desirable traits which may be useful for broadening the gene pool. *Ribes nigrum* cultivars have a range of descriptive characters (Table 2).

1.1.3.2 Red Currants

Ribes, the red currant subgenus, has crystalline glands on young growth (Brennan 1996). Several species have economic importance: selections of *R. sativum* Syme (+ *R. vulgare* Jancz.) were initially made from native stands in northwestern Europe. *Ribes petraeum* Wulf., a montane species, was also selected from the wild, while most of the cultivated red currants were derived from *R. rubrum* L., a Scandinavian species which is native as far north as 70°N latitude (Brennan 1996). *R. rubrum* is an unarmed shrub that grows to 2 m (Rehder 1986). The shoots are glabrous or have glandular hairs. Stems are covered with a smooth pale yellow bark. The leaves are deeply cordate, 3- to 5-lobed, 6×7 cm in diameter. Flowers, which occur on long racemes, are greenish tinged with purple. The hypanthium is almost flat and the petals are very small. The fruit is globose, 6–10 mm in diameter, red and glabrous.

Ribes triste Pallas is the North American red currant, with similar fruit quality to European red currants, but not developed for cultivation. Two additional species, *Ribes spicatum* Robs. from Norway and *R. multiflorum* Kit. from England, are used in red currant genetic improvement programs. White and pink currants are a color form of the red species.

1.1.3.3 Gooseberries

Gooseberry species have nodal spines. The European gooseberry, *R. uva-crispa* L. (+ *R. grossularia* L.), native in the United Kingdom eastward through northern Europe, the Caucasus, and North Africa, is most frequently selected for cultivar development (Brennan 1996). *R. uva-crispa* is a spiny shrub that grows as high as 1.5 m tall (Liberty Hyde Bailey Hortorium 1976). Stems have two to three spines at the nodes. Leaves are as large as 5 × 6.5 cm, sparsely pubescent or glabrous. Flowers occur in axillary clusters of one to three (much fewer than those on currant racemes), are pale green, sometimes pinkish, and have a hemispherical hypanthium, reflexed sepals, and short white petals. The fruit, which can be hispid, is globose to ovoid, about 10 mm in diameter, green, yellow, or purplish-red.

American species, such as *R. divaricatum* Dougl., *R. hirtellum* Michx and *R. oxyacanthoides* L., are used extensively in breeding with the European gooseberry. In England in the late 18th to 19th centuries, groups of amateur growers formed organizations with the purpose of increasing gooseberry fruit

Table 2. Descriptive characteristics of selected *Ribes nigrum* L. black currant cultivars

Cultivar	Country of origin	Vigor	Habit	Yield	Vitamin C	Flowering season	Cold hardy	Spring frost	Rust
Baldwin	United Kingdom	2	3	2	3	2	1	1	1
Beloruskaya sladkaya	Belarus	2	3	2	3	2	3	3	2
Ben Alder	United Kingdom	2	3	2	2	3	1	2	1
Ben Lomond	United Kingdom	2	3	2	3	3	1	2	2
Ben Tirran	United Kingdom	3	3	2	3	3	1	2	2
Blackdown	United Kingdom	3	3	3	2	2	1	1	1
Boskoop Giant	The Netherlands	3	2	2	3	1	2	2	1
Brodtopp	Finland	2	1	2	2	1	3	3	1
Consort	Canada	2	3	2	2	1	2	2	3
Crandall (<i>R. odoratum</i>)	United States	3	2	3	1	3	2	2	3
Crusader	Canada	2	2	2	2	1	2	2	3
Golubka	Russian Federation	2	2	2	3	2	3	3	1
Minaj Smyriov	Belarus	2	2	2	2	2	3	3	2
Noir de Bourgogne	France	3	2	3	2	1	1	2	1
Ojebyn	Sweden	2	2	2	1	2	3	3	1
Pilot A. Mamkin	Russian Federation	2	2	2	2	2	3	3	2
StorKlas	Sweden	2	1	2	1	1	3	3	1
Silvergieters Zwarte	Germany	3	3	2	nd	1	2	2	1
Titania	Sweden	3	2	3	2	1	3	3	3
Wellington XXX	United Kingdom	3	3	1	2	1	1	1	1

Vigor¹: 1 = least, 3 = most; Habit¹: 1 = spreading, 3 = erect; Yield¹: 1 = least, 3 = most; vitamin C in fruit²: 1 = low, 3 = high; Flowering season¹: 1 = early, 3 = late; Cold hardy²: 1 = least, 3 = most; Spring frost resistance²: 1 = least, 3 = most; White pine blister rust, *Cronartium ribicola* Fisch., (rust) resistance¹: 1 = least, 3 = most.

¹ Data collected at the USDA-ARS National Clonal Germplasm Repository, Corvallis, Oregon.

² Adapted from Brennan (1996) and Tuinyla and Lukosevicius (1996).

size and improving quality. These groups were quite successful and the crop thrived until the introduction of American powdery mildew, *Sphaerotheca mors-uvae* (Schw.) Berk. The gooseberry cultivars of the time were quite susceptible and acreage was greatly reduced (Brennan 1996).

1.1.3.4 Hybrids

Ribes x nidigrolaria Bauer is a hybrid cross of black currants with gooseberries (Brennan 1996). These man-made hybrids, commonly referred to as jostaberries, are very vigorous, do not have the acrid odor of black currants, have no or reduced spines, and are disease-resistant. These cultivars are grown more by homeowners rather than commercial growers. Their disease resistance and large size fruit are popular with organic farmers.

1.1.3.5 Ornamentals

Ornamental and flowering *Ribes* species have a broad range of colors. The American species, *R. aureum* Pursh and *R. odoratum* Wendl., have fragrant yellow flowers with tubular hypanthia that bloom in spring (Rehder 1986). The fruits are black but do not have the "black currant odor" characteristic of *R. nigrum*. Another American species, *R. sanguineum* Pursh, has a range of flower color variants from white to dark red-purple, and is used in landscape plantings for spring bloom and wildlife habitat. Unfortunately, this species tends to be susceptible to white pine blister rust (Hummer and Finn 1999). Some of the American gooseberry species, such as *R. speciosum* Pursh., *R. lobbii* Gray, and *R. menziesii* Pursh, have very attractive fuchsia-like flowers and are planted for their ornamental landscape attributes (Brennan 1996).

1.1.3.6 Endangered Species

The genus *Ribes* is fairly robust. Most species are broadly distributed and are not in danger of extinction. However, the World Conservation Monitoring Center 1997 *Red List of Threatened Plants* (www.wcmc.org) includes 18 *Ribes* species. *Ribes kolymense* (Trautv.) Komarov ex Pojark is extinct from the former Soviet states; three American and one Sardinian species are endangered; six American are vulnerable; two from the Pacific Northwest, another Sardinian and a Chilean species are rare; three Russian species are indeterminate (Table 3). *Ribes ussuriense*, one of the Russian species listed as indeterminate, contains the dominant gene, *Cr*, for immunity from white pine blister rust (Brennan 1996). Genes from this species have allowed the cultivation of black currants in white pine blister rust restricted zones of the United States.

The Endangered Species Act of the United States (Department of the Interior, Fish and Wildlife Service, 50 CFR, Part 17) lists the Miccosukee gooseberry, *R. echinellum* (Cov.) Rehder (Table 3). This spiny-fruited gooseberry species, whose native habitat occurs along the shoreline of Lake Miccosukee near Monticello, Florida, and in limited locations in South Carolina

Table 3. Threatened *Ribes* species

Species	Locality	Source	Designation
<i>R. amaranum</i> Munz var. <i>hoffmannii</i> Munz	California, United States	WCMC	V
<i>R. armenum</i> Pojark.	Armenia	WCMC	I
<i>R. binominatum</i> Heller	California and Oregon, United States	WCMC	V
<i>R. canthariforme</i> Wiggins	California, United States	WCMC	V
<i>R. divaricatum</i> (Heller) Jepson var. <i>Parishii</i> (Heller) Jepson	California, United States	WCMC, ONHP	V
<i>R. cereum</i> var. <i>colubrinum</i> Hitchc.	Oregon, United States	ONHP	R
<i>R. echinellum</i> (Coville) Rehd.	Florida, Georgia, South Carolina, United States	WCMC, ESA	E
<i>R. erythrocarpum</i> Coville & Lieb.	Oregon, United States	WCMC	V
<i>R. integrifolium</i> Philippi	Chile	WCMC	R
<i>R. klamathense</i> (Cov.) Fedde	Oregon, United States	ONHP	I
<i>R. kolymense</i> (Trautv.) Komarov ex Pojark	former Soviet States	WCMC	EX
<i>R. malvifolium</i> Pojark.	Tajikistan, Uzbekistan	WCMC	I
<i>R. menziesii</i> Pursh var. <i>thacherianum</i> Jepson	California, United States	WCMC	E
<i>R. niveum</i> Lindl.	Idaho, Nevada, Oregon, and Washington, United States	WCMC	R
<i>R. oxyacanthoides</i> L. subsp. <i>irriguum</i> (Dougl.) Sinnott	British Columbia, Canada; Idaho, Montana, Oregon, and Washington, United States	WCMC	R
<i>R. sandaliticum</i> (Arrigoni) Arrigoni	Sardinia	WCMC	R
<i>R. sardoum</i> Martelli	Sardinia	WCMC	E
<i>R. sericeum</i> Eastw.	California, United States	WCMC	V
<i>R. tularense</i> (Coville) Fedde	California, United States	WCMC	V
<i>R. ussuriense</i> Jancz.	Russian Federation	WCMC	I

WCMC = World Conservation Monitoring Center, Red List of Threatened Plants, www.wcmw.org; ESA Endangered Species Act of the United States, Department of the Interior, Fish and wildlife Service, 50 CFR Part 17; ONHP = Oregon Natural Heritage Program (www.abi.org/nhp). Designations are: EX = extinct, E = endangered, R = rare, V = vulnerable, I = indeterminate.

and Georgia, is threatened by encroaching human development. Several accessions of this species are maintained *ex situ* at the National Clonal Germplasm Repository at Corvallis, Oregon. The Oregon National Heritage Program lists *R. cereum* var. *colubrinum* Hitchc. as rare and *R. divaricatum* and *R. klamathense* (Cov.) Fedde as indeterminate (Table 3).

1.2 Various Methods for the Storage of *Ribes* Germplasm

Ribes germplasm collections are maintained as plants in fields or screened houses to maintain clonal identities. Alternatively, they may be held as *in vitro* cultures or cryopreserved as shoot tips in liquid nitrogen. Species collections may be held as seed stored at -20°C , -80°C , or -196°C .

1.2.1 Propagation and Culture of *Ribes* Plants

Currants root readily from dormant stem cuttings taken in the fall, or softwood taken in the spring. Most black currants root well from any type of cutting, but red currants do not root as easily (Brennan 1996). Nurseries in milder climates, such as the Pacific Northwest of the United States, cut 15-cm-long stems of black currant cultivars in late October and cover the lower third of the stem with soil. These remain in the field throughout the winter while roots form. Rooted cuttings are dug and shipped in the early spring.

Gooseberries, particularly cultivars derived from European species, root less readily than currants. Generally, a basal application of auxin is needed for successful rooting of hardwood cuttings. In cases where cuttings will not root, mound layering or grafting must be attempted. Budding or whip-and-tongue grafting, with similar procedures to those used for temperate fruit trees, can be performed in the dormant season. Clones of *R. aureum* or *R. odoratum* have been selected for rootstocks (Hamat et al. 1989).

1.2.2 Cold Storage of *In Vitro* Cultures

Cold storage of *in vitro* cultures of *Ribes* germplasm was first reported by Gunning and Lagerstedt (1985) who found that plant condition declined within a few months for *Ribes* accessions stored at 5°C but plantlets remained in excellent condition with storage at -1°C . Brennan et al. (1990) stored three *Ribes* cultivars *in vitro* at 6°C for 3 months with good survival and morphogenic potential. Reed and Chang (1997) reported data for 80 *Ribes* genotypes in dark storage at 4°C with a mean storage time of 1.4 years and 40 accessions at -1°C in the dark with a mean storage time of 2.76 years. The addition of a 12-h photoperiod improved the 4°C storage mean to 2.4 years making it nearly equivalent to the -1°C storage. *In vitro* cultures of over 100 *Ribes* genotypes are preserved in the collection at NCGR-Corvallis (Reed and Chang 1997).

1.2.3 Cryopreservation

Early cryopreservation studies of *Ribes* species and cultivars involved evaluation of freezing survival of dormant plant buds in mid-winter. Sakai and Nishiyama (1978) obtained excellent results when testing dormant buds of 'Oregon Champion' gooseberry and 'London Market' currant for survival following exposure to liquid nitrogen (LN). Dormant buds were frozen at 5°C/day to -40°C, plunged in LN, and thawed slowly with 100% survival. Reed and Yu (1995) cryopreserved shoot tips from in vitro grown *Ribes aureum*, *R. diacantha* Pall., and *R. rubrum* plants using a variety of techniques. Controlled cooling and vitrification were successful for two of three genotypes while encapsulation-dehydration worked well for all three. Benson et al. (1996) studied *R. nigrum* cultivars cryopreserved by the same three methods as Reed and Yu (1995). Some living plants were recovered with all three techniques, but encapsulation-dehydration was the most successful, followed by vitrification. Controlled cooling resulted in low viability in these tests. These studies also found that cold acclimatization and dimethyl sulfoxide (DMSO) pretreatments did not influence the ice nucleation and melt characteristics of the apices as determined by differential scanning calorimetry (DSC). Improved recovery of vitrified currant (*R. aureum* and *R. ciliatum* Humb. & Bonpl.) shoot tips and callus was obtained following a 2-h pretreatment in sucrose, proline, abscisic acid responsive proteins (RABP) or bovine serum albumin (BSA) (Luo and Reed 1997). A 1% BSA pretreatment was suggested as the most economical and available of the materials tested and increased shoot tip regrowth of some *Ribes* that had proved difficult to cryopreserve from 40% to nearly 70% following vitrification. Two *Ribes nigrum* cultivars, Ojebyn and Ben Lomond, cryopreserved by the encapsulation-dehydration technique, were successfully conditioned on a 0.75M sucrose medium and produced recovery equivalent (90–100% regrowth of meristems) to those cold acclimated for 1 week (Dumet et al. 2000). Both vitrification and encapsulation-dehydration methods were shown to be suitable for storage of *Ribes* germplasm in international genebanks, although some differences in results were noted between the two laboratories involved in the study (Reed et al. 2000).

2 Methodology/Protocol for Cold Storage and Cryopreservation

2.1 Initiation and Multiplication of In Vitro Cultures

Explants from potted greenhouse-grown plants were disinfected in 10% commercial bleach (sodium hypochlorite 0.5%) with 5 drops of Tween 20 per 500ml, shaken on a rotary shaker for 10 min, and rinsed 3 × in sterile, deionized water. Single node sections were transferred to 16 × 100mm tubes with

10ml of liquid Murashige and Skoog (MS; Murashige and Skoog 1962) medium (pH6.9) with no growth regulators for contaminant detection (Reed et al. 1995). Uncontaminated explants were transferred into Magenta GA7 (Magenta, Chicago IL) boxes with 40ml of NCGR-*Ribes* (RIB) medium and subcultured at 3-week intervals.

Micropropagated plantlets were multiplied on NCGR-RIB, which contains MS mineral salts and vitamins, but with only 30% of the normal ammonium and potassium nitrate concentrations, and (per liter): 50mg ascorbic acid, 20g glucose, 0.1mg N6-benzyladenine (Sigma Chemical, St. Louis, MO), 0.2mg GA3, 3.5g agar (Bitec, Difco, Detroit, MI) and 1.45g Gelrite (Kelco, San Diego, CA) at pH5.7. Plants were grown at 25°C with 16-h days ($25\mu\text{molm}^{-2}\text{s}^{-1}$).

2.2 Cold Storage of *Ribes* In Vitro Cultures

2.2.1 General Growth Conditions

Micropropagated plantlets were multiplied in Magenta GA7 boxes on NCGR-RIB as noted above. Plantlets were divided, transferred to fresh medium at 3-week intervals, and indexed for bacterial contaminants before storage (Reed and Tanprasert 1995; Reed et al. 1995).

2.2.2 Cold Storage of in Vitro Plantlets

Ten plantlets (2.5–3 cm) of each genotype were transferred to individual chambers of 5-chamber Star-pak bags (Gardner Enterprises, Willis, TX). Each chamber contained 10ml hormone-free NCGR-RIB with 3.5g agar and 1.75 Gelrite (equivalent to 8g agar). Bags were sealed twice with an impulse sealer, labeled, returned to the growth room for 1 week, and then cold acclimatized (CA) for 1 week (8-h days at 22°C and 16-h nights at –1°C). Storage was at –1°C in the dark or 4°C with or without a 12-h photoperiod ($3\mu\text{molm}^{-2}\text{s}^{-1}$). Each shoot was evaluated at 3- to 4-month intervals. Shoots were rated on a vigor scale of 0 to 5, based on plant appearance: 5 + dark green leaves and stems, no etiolation; 4 + green leaves and stems, little etiolation; 3 + shoot tips and upper leaves green, some etiolation, 2 + shoot tips green, leaves and stems mostly brown, base may be dark brown, should be removed for subculture; 1 + plant mostly brown, only extreme shoot tip green, much of base dark brown; 0 + plant totally brown, no visible green on shoot tip, dead. A completely randomized design was used for the cold storage experiments. Data were analyzed for ANOVA using the factor program on MSTATC (1988).

2.3 Cryopreservation of *Ribes* Shoot Tips

2.3.1 General Growth Conditions

Micropropagated plantlets were multiplied and shoot tips recovered on NCGR-RIB under the growth conditions described above. All cultures were CA for one week (as noted above) before 0.8mm shoot tips were excised. Additional CA may be useful for some genotypes as was found for pears and blackberries that exhibited low regrowth following 1-week CA and cryopreservation but high regrowth with 4–10 weeks CA (Chang and Reed 2000).

2.3.2 Protocol Optimization Experiments

2.3.2.1 Controlled Cooling

The method used was developed for pear (Reed 1990) and further modified for *Ribes* (Reed and Yu 1995). Shoot tips excised from CA plantlets were CA in the incubator for 2 days on NCGR-RIB with 5% DMSO and additional Gelrite (0.3 g/l) in the medium, then transferred to 0.25 ml liquid NCGR-RIB in 1.2-ml plastic cryotubes and 1 ml of the cryoprotectant PGD [10% each polyethylene glycol (MW 8000), glucose and DMSO in NCGR-RIB liquid medium] was added over 30 min. A 30-min equilibration at 4°C was followed by cooling at 0.1 or 0.3°C/min to –40°C and plunging into LN. Samples were thawed for 1 min in 45°C water then transferred to 22°C water for 2 min, rinsed in liquid NCGR-RIB and plated on NCGR-RIB for recovery.

2.3.2.2 Encapsulation-Dehydration

This method was developed for pear (Dereuddre et al. 1990) and further adapted for *Ribes* (Reed, unpubl.). CA shoot tips were dissected onto agar plates then encased in alginate beads (3% low viscosity alginic acid with 0.75M sucrose) for 18-h pretreatment in liquid NCGR-RIB with 0.75M sucrose. Following pretreatment, beads were separated on sterile Petri dishes and air dried in the laminar flow hood for 4h, placed in cryotubes and plunged into LN. Vials were rewarmed at room temperature for 15 min. Vials were filled with liquid NCGR-RIB for 10 min to rehydrate the beads, then drained, and the encapsulated shoot tips were planted on NCGR-RIB recovery medium.

2.3.2.3 Vitrification

The vitrification technique for white clover (Yamada et al. 1991) was modified by Reed and Yu (1995) and further modified by Luo and Reed (1997). CA

shoot tips were pretreated for 2 days in a CA incubator (see general growth conditions) on NCGR-RIB with 5% DMSO and additional Gelrite (0.3 g/l). Shoot tips were removed from the pretreatment plates and placed in a cryotube with 1 ml of 1% bovine serum albumin (BSA) for 2 h at room temperature. The BSA solution was drawn off and PVS2 cryoprotectant (30% glycerol, 15% ethylene glycol and 15% DMSO in liquid NCGR-RIB with 0.4 M sucrose) was added to cryotubes on ice and stirred. After 20 min in PVS2, vials were submerged in LN. Samples were rewarmed for 1 min in 45 °C water, then transferred to 22 °C water for 2 min, and rinsed in liquid NCGR-RIB with 1.2 M sucrose before plating on NCGR-RIB recovery medium. One set of experiments compared the 5% DMSO pretreatment medium with 1.2 M sorbitol pretreatment medium but did not include the BSA presoak.

2.3.3 Sucrose Pretreatment as a Substitute for Cold Acclimatization

Ribes nigrum cvs. Ben Lomond and Ojebyn meristems were cryopreserved by encapsulation-dehydration (E-D) following the standard 1 week of cold acclimatization as listed above or 1 cm shoots with leaves removed were grown on NCGR-RIB with 0.75 M sucrose under standard light and temperature conditions (Dumet et al. 2000).

2.3.4 Pretreatment Effects on Vitrification Protocols

Pretreatments with 1–4 h immersion in 1, 5, 10 and 15% proline, 1 or 2% abscisic acid responsive proteins (RABP), 1% BSA, or 0.4 M sucrose in the standard NCGR-RIB were tested to determine their effectiveness on the standard vitrification protocol. After cold acclimatization and 48-h pregrowth on 5% DMSO medium, meristems were immersed in one of the solutions for 0–4 h, then it was removed, and vitrification solution was added followed by LN exposure, thawing, and regrowth. *Ribes ciliatum* Humb. & Bonpl. and *Ribes aureum* Pursh meristems and callus were used in this study (Luo and Reed 1997).

2.3.5 Thermal Analysis of Cryopreservation Protocols

Differential scanning calorimetry (DSC) was performed using a Perkin Elmer DSC7 and a TAC 7 PC (Benson et al. 1996). DSC profiles were constructed with a scanning rate of ± 10 °C/min. Zinc and indium standards were used for calibration. Samples of *Ribes nigrum* cvs. Ben Tron and Ben More were sealed in aluminum pans and scans were performed from +5 °C to –150 °C. Data from each cryopreservation technique included three cryovials. Recovery data was taken at 6 weeks. Cooling and warming profiles were constructed for each sample (3–6 replicates per technique).

2.3.6 Germplasm Storage Studies

A comparison of cryopreservation methods by separate laboratories using the same genotypes was initiated at NCGR in Corvallis, Oregon, USA, and the University of Abertay-Dundee, Dundee, Scotland. *Ribes nigrum* cv. Ojebyn and *Ribes rubrum* cv. Red Lake were cryopreserved using the three methods mentioned above (Reed et al. 2000).

2.4 Results

2.4.1 Cold Storage of *Ribes* In Vitro Cultures

Like many aspects of in vitro culture, plantlet response to cold storage is genotype dependent. Earlier work in this laboratory found 2.76 ± 0.7 years as the mean storage length for 40 *Ribes* accessions stored at -1°C in darkness for 15–24 months (Reed and Chang 1997). Storage at 4°C with a 12-h photoperiod produced results similar to the -1°C darkness ratings (Table 4). Dark 4°C storage required repropagation more than 1 year earlier (1.4 ± 0.8 year) than plantlets under the other two storage conditions. Ratings of plantlets stored at -1°C in the dark were similar to those at 4°C with a 12-h photoperiod. Individual genotypes varied greatly as indicated by the standard

Table 4. Storage duration of representative *Ribes* genotypes at the National Clonal Germplasm Repository, Corvallis, which remained viable (rated 2 or above) at 4°C with a 12 hr dim photoperiod

Genotype	Fruit type	Rating ^a	Months stored
Oregon	Gooseberry	2	21
Tsema	Black Currant	2	21
Kosmizenskaja	Black Currant	2	21
<i>R. curvatum</i>	Gooseberry	2	14
<i>R. curvatum</i>	Gooseberry	3	14
Rolan	Red Currant	2	14
Noire de Bourgogne	Black Currant	2	14
White Cherry	White Currant	2	14
Malling Redstart	Red Currant	2	13
Raby Castle	Red Currant	3	13
<i>R. viscosissimum</i>	Black Currant	3	13
Kerry	Black Currant	3	13
D. Young	Gooseberry	3	12
Malling Jet	Black Currant	2	12
Minj Smyriov	Black Currant	2	12
Baldwin	Black Currant	2	12

^a Reed, 1998 unpublished data. Shoots were rated on a vigor scale of 0 to 5, based on plant appearance: 5 = dark green leaves and stems, no etiolation; 4 = green leaves and stems, little etiolation; 3 = shoot tips and upper leaves green, some etiolation, 2 = shoot tips green, leaves and stems mostly brown, base may be dark brown, should be removed for subculture; 1 = plant mostly brown, only extreme shoot tip green, much of base dark brown; 0 = plant totally brown, no visible green on shoot tip, dead.

deviations of 9 to 10 months. The storage range for individual genotypes varied from 8 months to 3 years.

2.4.2 Cryopreservation of *Ribes* Shoot Tips

2.4.2.1 Protocol Optimization

Ribes cryopreservation protocols were optimized in several ways (Reed and Yu 1995; Reed et al. 2000). In the controlled cooling protocol regrowth of meristems exposed to cooling rates of 0.3°C/min and 0.5°C/min were not significantly different for three genotypes tested (Fig. 1). Pregrowth before vitrification in PVS2 on either sorbitol or DMSO media produced significant differences for two of three genotypes. DMSO was significantly better for two of the three and the third showed no difference (Fig. 1). Length of drying of alginate beads in the E-D method was significant for two of three genotypes with 3-h drying generally better than 2 h (Fig. 1). The success of the three cryopreservation methods tested on *Ribes* in vitro grown shoot tips varied greatly with the genotype of the accession (Fig. 1, Table 5). Overall, the encapsulation-dehydration method gave the highest survival for the genotypes tested. Vitrification techniques produced moderate to high recoveries for over half of the genotypes tested, and the addition of a 2-h presoak in BSA prior to PVS2 addition further improved recovery. Five genotypes were stored using the presoak-vitrification technique and are the beginning of a long-term *Ribes* base-germplasm collection held at the USDA-ARS National Seed Storage Laboratory, Fort Collins, Colorado. Control shoot tips (exposed to cryoprotectant but not LN) were recovered in high percentages (80–100%) for slow freeze and encapsulation-dehydration techniques. Vitrification solutions were more toxic to the control shoot tips and reduced the percentage of regrowth (60–80%). Shoot tips produced from surviving meristems developed into normal plantlets (Fig. 2).

Table 5. Regrowth of *Ribes* meristems following 1-hr exposure to LN. Methods used were controlled cooling at 0.3°C/min (CC), vitrification in PVS2 (Vit), and encapsulation-dehydration with a 3 hr dehydration (E-D)

Genotype	Control	CC	Vit	E-D
<i>R. rubrum</i> cv. Cherry	80–100% ^a	30%	10%	60%
<i>R. diacantha</i>	80–100% ^a	70%	70%	60%
<i>R. aureum</i>	80–100% ^a	55%	60%	90%
<i>R. nigrum</i> cv. Ben More	70–80% ^b	10%	20%	80%
<i>R. nigrum</i> cv. Ben Tron	100% ^b	–	60%	80%
<i>R. aureum</i> cv. Bronze	80% ^c	–	44%; 69% ^d	–
<i>R. ciliatum</i>	80% ^c	–	42%; 68% ^d	–

^a Reed and Yu, 1995.

^b Benson et al., 1996.

^c Luo and Reed, 1997.

^d standard method; with BSA presoak.

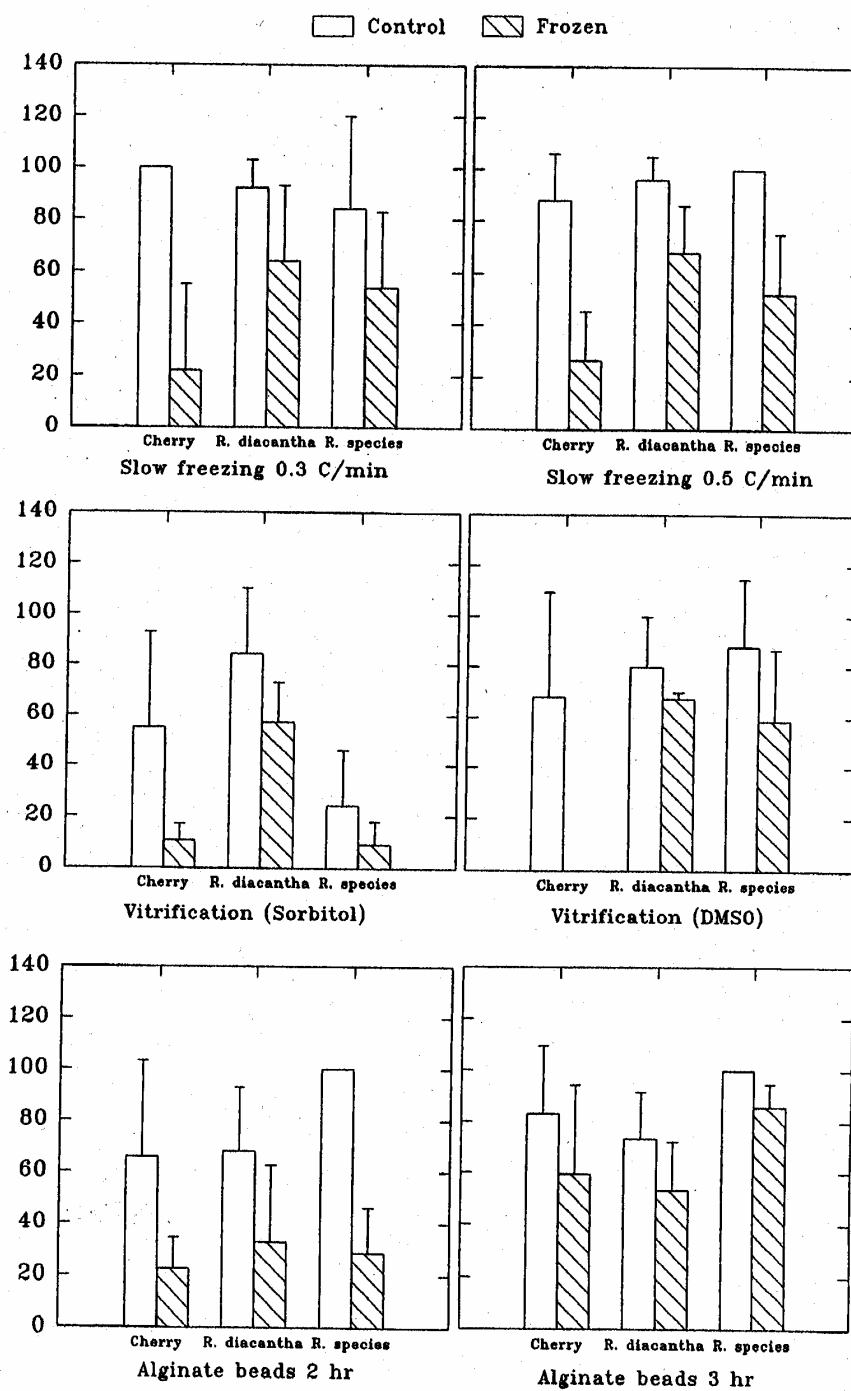


Fig. 1. Regrowth of control and frozen meristems of three *Ribes* genotypes following cryoprotection or cryoprotection and freezing with six different techniques (mean \pm SD). Controlled cooling (*slow freezing*) at 0.3 or 0.5°C/min; vitrification with a 1.2M sorbitol pretreatment (*sorbitol*); or 5% DMSO pretreatment (*DMSO*); Encapsulation-dehydration with 2- or 3-h dehydration (alginate beads). *Ribes rubrum* cv. Cherry, *R. diacantha* and *R. aureum* (species). (Data from Reed and Yu 1995)

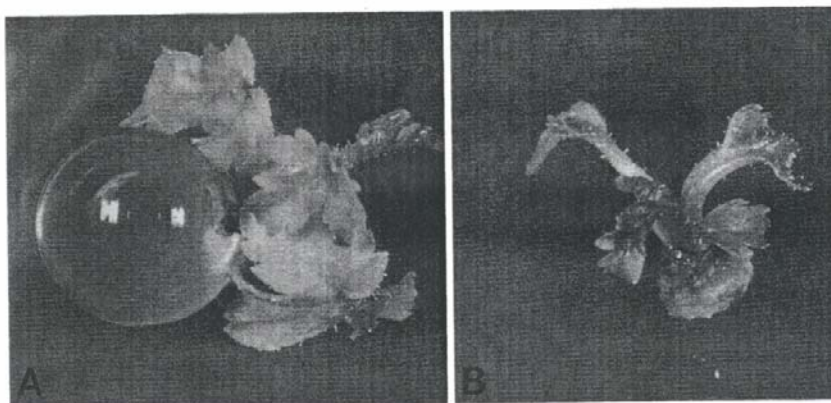


Fig. 2. Shoots regrown from cryopreserved *Ribes nigrum* cv. Ojebyn shoot tips by **A** alginate encapsulation-dehydration or **B** vitrification in PVS2 cryoprotectant. (Photographs courtesy of Rex Brennan, Scottish Crop Research Institute)

Table 6. Effect of 2-hr pretreatments on the survival of vitrified *Ribes aureum* and *R. ciliatum* meristems and callus 4 weeks after warming: 0.4M sucrose in liquid medium (0.4M sucrose in NCGR-RIB) alone or with 5% proline, 1% abscisic acid responsive protein (RABP), or 1% bovine serum albumin (BSA)

Genotype	Control	0.4M Sucrose	5% Proline	1% RABP	1% BSA
<i>Meristems</i>					
<i>R. aureum</i>	44.0 ± 1.0 ^c	52.7 ± 1.0 ^b	64.7 ± 1.3 ^a	66.0 ± 1.3 ^a	68.7 ± 1.5 ^a
<i>R. ciliatum</i>	42.1 ± 0.0 ^c	53.8 ± 1.7 ^b	67.4 ± 2.4 ^a	76.3 ± 7.0 ^a	67.6 ± 2.5 ^a
<i>Callus</i>					
<i>R. aureum</i>	39.8 ± 0.5 ^c	45.8 ± 0.4 ^b	50.6 ± 0.8 ^a	51.0 ± 0.6 ^a	50.6 ± 0.5 ^a
<i>R. ciliatum</i>	39.9 ± 2.1 ^c	48.7 ± 1.2 ^b	61.0 ± 0.8 ^a	64.3 ± 1.9 ^a	64.2 ± 2.2 ^a

Percentage survival data were transformed by arcsine square root and expressed as means ± SEM%. Means separation by Duncan's multiple range test ($p \leq 0.05$) (for meristems $n = 60$; for callus $n = 45$). Values in a row with different letters are significantly different. (Luo and Reed 1997).

2.4.2.2 Pretreatment Effects on Vitrification Protocols

Vitrified meristems and callus both exhibited improved recovery following vitrification when pretreated for 2h with sucrose, proline, RABP, or BSA. Immersion for 2h was better than longer or shorter intervals. Immersion for 2h in 0.4M sucrose solution significantly improved regrowth of callus and meristems of both species (Table 6). Pretreatment of meristems and callus with 5% or 10% proline, 1% RABP, or 1% BSA in 0.4M sucrose NCGR-RIB all had significantly better results than the sucrose medium alone (Table 6). Shoots grew directly from meristems without a callus phase. Pretreated meristems resumed growth 3 days after warming and reached maximum regrowth by 1 week, compared with 2 weeks for controls (Luo and Reed 1997).

2.4.2.3 Sucrose Pretreatment as a Substitute for Cold Acclimatization

Both *R. nigrum* cultivars tested had high regrowth following encapsulation-dehydration (93–100%) and LN exposure (90–100%). There were no significant differences between cultivars or between the cold acclimatized meristems and those conditioned for 1 week on NCGR-RIB with 0.75 M sucrose (Dumet et al. 2000).

2.4.2.4 Thermal Analysis

Differential scanning calorimetry profiles showed highly reproducible ice nucleation and melting phenomena (Benson et al. 1996). PVS2 solution produced nucleation and melt profiles; however, profiles of vitrified meristems with the PVS2 solution removed showed no evidence of nucleation on cooling or warming indicating that the glass associated with the tissues is stable on warming. Analysis of dehydration treatments demonstrated reproducible ice nucleation and melt events associated with a major thermal peak. The stability of the glassy state on warming appeared marginal even after 4 h of dehydration before LN exposure (data not shown).

2.4.2.5 Germplasm Storage Studies

Some differences were evident between the two laboratories, so more precise utilization of protocols may be necessary to standardize the procedures. Encapsulation-dehydration produced excellent results for both genotypes at both locations (<90% regrowth). Vitrification was moderately successful at both locations for both genotypes (25–68% regrowth). Controlled freezing produced little regrowth from either genotype, perhaps due to differences in controlled rate freezers. Differences in familiarity with the process between the two laboratories may also have influenced the results (Reed et al. 2000).

2.5 Discussion

2.5.1 *In Vitro* Cold Storage

Cold storage at -1°C in darkness or 4°C with a 12-h photoperiod was successfully used as an intermediate-length germplasm conservation method. Most genotypes stored under these conditions were held for over 2 years without repropagation and recovered quickly when placed in growth room conditions on fresh culture medium. The cold hardiness of most *Ribes* genotypes made them excellent candidates for this form of intermediate storage. Cold storage of many temperate crops is best at -1 to 4°C (Reed and Chang 1997). Differences in storage time among accessions may be due to the condition of the plant when stored, size of propagule, preparation of medium, or placement of the plant in the medium. Storage consistency depends on the

technical skills of laboratory workers. *Ribes* shoots in cold storage are particularly sensitive to size variation; small shoots store for shorter periods than do intermediate or large shoots or plantlets. Genotypes with a short, bushy growth habit exhibit a shorter storage duration than those that are more elongated (data not shown). In addition to their adaptability to medium-term storage, in vitro *Ribes* shoots are ideal for germplasm exchange. Shoots can be removed from cold storage and shipped to requestors as needed. In vitro cultures are often preferred for international shipments because many, although not all, disease and insect threats are eliminated when the plants are initiated into culture.

2.5.2 Cryopreservation

Seeds cannot be used as a base (long term) storage collection to preserve specific *Ribes* genotypes, and alternative clonal methods must be employed. Researchers can now recover 60–100% of cryopreserved shoot tips, indicating that cryopreservation is an acceptable form of long-term storage for clonal *Ribes* germplasm. Preliminary screening indicates that most genotypes can be successfully cryopreserved using cold acclimation and the vitrification method with presoak or the encapsulation-dehydration methods. Benson et al. (1996) showed that devitrification of PVS2 can occur on warming of vials following exposure to liquid nitrogen, thus reducing the survival of shoot tips. The addition of a 2-h presoak in proline or BSA to the vitrification protocol greatly improved survival of *Ribes* shoot tips (Luo and Reed 1997). Immersion for 2 h in sucrose improved recovery of meristems and callus; however, extended immersion was detrimental. Extended immersion may cause overdehydration of the cells resulting in injury unrelated to the effects of low temperature. Proline, BSA and RABP 2-h immersion greatly improved recovery of both callus and meristems. These substances may have provided additional stability to the glassy state of the cryopreserved tissues. An unanticipated result of the pretreatment protocols was the rapid recovery of meristems in almost all pretreatment groups. Cryoprotectant injury to *Ribes* meristems is common with highly concentrated vitrification solutions such as PVS2 but was overcome with these pretreatments (Luo and Reed 1997). For encapsulated shoot tips adequate desiccation was needed to maintain the stability of the glassy state (Benson et al. 1996). A sucrose-preconditioning step can substitute for the cold-acclimatization period prior to the encapsulation-dehydration process for some *Ribes* genotypes (Dumet et al. 2000). The increased tolerance to LN could be due to massive absorption of sucrose by the cells, or the result of osmotic stress. Standardization of techniques between laboratories is needed if cryopreservation is to be instituted on a large scale. The first attempt at this standardization indicated that transfer of techniques between laboratories can be successful. These studies indicate that some protocols are more easily transferred than others, and success with a protocol can be linked to specific portions of each process. When transferring protocols to other labo-

ratories, training of personnel, standardization of growth rooms and equipment, and clearly written step-by-step protocols for each technique are key points to consider (Reed et al. 2000). Long-term *Ribes* germplasm storage has been initiated at NCGR, Corvallis, Oregon (PVS2 vitrified meristems) and at the University of Abertay-Dundee in Scotland (encapsulated meristems) (Reed et al. 2000).

3 Summary and Conclusions

Active-clonal field collections of *Ribes* germplasm can now be secured with secondary (active-backup) in vitro collections and cryopreserved (base) collections. Recent advances in the cold storage of *Ribes* in vitro cultures and cryopreservation make both techniques appropriate for germplasm conservation. Cold storage at -1°C in darkness or 4°C with a 12-h photoperiod is successful for medium-term storage of 2–3 years and allows availability for germplasm exchange. Cryopreserved collections of *Ribes* germplasm in liquid nitrogen were recently initiated at the National Clonal Germplasm Repository and the University of Abertay-Dundee. These in vitro techniques, when used in concert with active field collections, constitute a reliable system for conserving *Ribes* genetic resources.

References

- Benson EE, Reed BM, Brennan RM, Clacher KA, Ross DA (1996) Use of thermal analysis in the evaluation of cryopreservation protocols for *Ribes nigrum* L. germplasm. *Cryo Lett* 17: 347–362
- Berger A (1924) A taxonomic review of currants and gooseberries. *Bull NY State Agric Exp Sta* 109
- Brennan RM (1996) Currants and gooseberries. In: Janick J, Moore JN (eds) *Fruit breeding*, vol II. Vine and small fruit crops. Wiley, New York, chap 3, pp 191–295
- Brennan RM, Millam S, Davidson D, Wilshin A (1990) Establishment of an in vitro *Ribes* germplasm collection and preliminary investigations into long-term low temperature germplasm storage. *Acta Hort* 280:109–112
- Chang Y, Reed BM (2000) Cold acclimatization improves the cryopreservation of in vitro-grown *Pyrus* and *Rubus* meristems. In: Engelmann F, Takagi H (eds) *Cryopreservation of tropical plant germplasm. Current research progress and application*. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan/International Plant Genetic Resources Institute, Rome, Italy, pp 382–384
- Coville FV, Britton NL (1908) *Grossulariaceae*. *North Am Flora* 22:193–225
- Cronquist A (1981) *An integrated system of classification of flowering plants*. Columbia Univ Press, New York
- Darlington CD (1929) A comparative study of the chromosome complement in *Ribes*. *Genetica* 11:267–269
- de Janczewski E (1907) *Monograph of the currants Ribes L.* (in French). *Mem Soc Phys Hist Nat Geneve* 35:199–517

- Dereuddre J, Scottez C, Arnaud Y, Duron M (1990) Effects of cold hardening on cryopreservation of axillary pear (*Pyrus communis* L. cv. Beurre Hardy) shoot tips of in vitro plantlets. *CR Acad Sci Paris* 310:265–272
- Dumet D, Chang Y, Reed BM, Benson EE (2000) Replacement of cold acclimatization with high sucrose pretreatment in black currant cryopreservation. In: Engelmann F, Takagi H (eds) Cryopreservation of tropical plant germplasm. Current research progress and application. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan/International Plant Genetic Resources Institute, Rome, Italy, pp 385–387
- Engler A, Prantl K (1891) *Ribesioideae*. *Naturl Pflanzenfam* 3:97–142
- FAO (1999) Food Agriculture Organization of the United Nations, World Wide Web Statistical Database. www.fao.org
- Gunning J, Lagerstedt HB (1985) Long-term storage techniques for in vitro plant germplasm. *Proc Int Plant Prop Soc* 35:199–205
- Hamat L, Porpaczy A, Himelrick DG, Galletta GJ (1989) Currant and gooseberry management. In: Galletta GJ, Himelrick DG (eds) *Small fruit crop management*. Prentice Hall, New York, pp 245–272
- Hummer K (2000) History of the origin and dispersal of white pine blister rust. *HortTechnology* 10:515–517
- Hummer K, Finn C (1999) Third year update: *Ribes* susceptibility to white pine blister rust. *Acta Hort* 505:403–408
- Jennings DL, Anderson MM, Brennan RM (1987) Raspberry and blackcurrant breeding. In: Abbot AJ, Atkin RK (eds) *Improving vegetatively propagated crops*. Academic Press, London, pp 135–147
- Keep E (1962) Interspecific hybridization in *Ribes*. *Genetica* 33:1–23
- Komarov VL (ed) (1971) Flora of the [former] USSR, vol IX. In: *Ribesioideae* Engl (translated from the Russian by the Israel Program for Scientific Translation, Jerusalem). Keter, London, pp 175–208
- Lamarck JB, De Candolle AP (1805) *Flore francaise*. Desray, Paris
- Liberty Hyde Hortorium (1976) *Hortus third*. Macmillan, New York, pp 969–971
- Luo J, Reed BM (1997) Absciscic acid-responsive protein, bovine serum albumin, and proline pretreatments improve recovery of in vitro currant shoot tips and callus cryopreserved by vitrification. *Cryobiology* 34:240–250
- Messinger W, Liston A, Hummer K (1999) *Ribes* phylogeny as indicated by restriction-site polymorphisms of PCR-amplified chloroplast DNA. *Plant Syst Evol* 217:185–195
- MSTATC (1988) MSTATC – a software program for the design, management, and analysis of agronomic research experiments. Michigan State University, East Lansing, Michigan
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
- Reed BM (1990) Survival of in vitro-grown shoot tips of *Pyrus* following cryopreservation. *HortScience* 25:111–113
- Reed BM, Chang Y (1997) Medium- and long-term storage of in vitro cultures of temperate fruit and nut crops. In: Razdan MK, Cocking EC (eds) *Conservation of plant genetic resources in vitro*, vol 1. Science Publishers, Enfield, New Hampshire
- Reed BM, Tanprasert P (1995) Detection and control of bacterial contaminants of plant tissue cultures. A review of recent literature. *Plant Tissue Culture Biotech* 1:137–142
- Reed BM, Yu X (1995) Cryopreservation of in vitro-grown gooseberry and currant shoot tips. *Cryo Lett* 16:131–136
- Reed BM, Buckley PM, DeWilde TN (1995) Detection and eradication of endophytic bacteria from micropropagated mint plants. *In Vitro Cell Dev Biol* 31P:53–57
- Reed BM, Brennan RM, Benson EE (2000) Cryopreservation: an in vitro method for conserving *Ribes* germplasm in international genebanks. In: Engelmann F, Takagi H (eds) *Cryopreservation of tropical plant germplasm. Current research progress and application*. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan/International Plant Genetic Resources Institute, Rome, Italy, pp 470–472
- Rehder A (1986) *Manual of cultivated trees and shrubs*, 2nd rev and enlarged edn. Dioscorides Press Portland, Oregon, pp 293–311

- Sakai A, Nishiyama Y (1978) Cryopreservation of winter vegetative buds of hardy fruit trees in liquid nitrogen. *HortScience* 13:225-227
- Sinnott QP (1985) A revision of *Ribes* L. subg. *Grossularia* (Mill.) per. Sect. *Grossularia* (Mill.) Nutt. (Grossulariaceae) in North America. *Rhodora* 87:189-286
- Tuinyla V, Lukosevicius A (1996) Pomology of Lithuania. Lithuanian Science and Encyclopedia Publisher; Vilnius Lietuva, Lithuania
- Vetenant (1799) Tableau du regne vegetal. Drisonnier, Paris
- Yamada T, Sakai A, Matsumura T, Higuchi S (1991) Cryopreservation of shoot tips of white clover (*Trifolium repens* L.) by vitrification. *Plant Sci* 78:81-87
- Zielinski QB (1953) Chromosome numbers and meiotic studies in *Ribes*. *Bot Gaz* 114:265-274